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Liquid Smoke Toxicity Properties of Production of Raw Materials with Variation of Temperature and Concentration of Different

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Abstract : This study aims to determine the nature of the toxicity of liquid smoke derived from a combination treatment of the raw material with temperature levels and different concentrations of liquid smoke. This study was conducted experimentally using a completely randomized factorial design 3 x 4 x 6 with three replications thus obtained 72 experimental units. A factor is the type of raw material comprising coconut husk, coconut shell and cinnamon, factor B level pyrolysis temperature of 100±10°C; 200±10 °C; 300±10°C; and 400±10°C and factor C level liquid smoke concentration is 0 ppm, 12.5 ppm, 25 ppm, 50 ppm, 100 ppm to 500 ppm and 1000 ppm. The parameters were observed consisting of liquid smoke toxicity properties consisting of Artemiasalina leach mortality percentage in the form of probit. Results showed there was an interaction ($P < 0.05$) between use kinds of raw materials to the pyrolysis temperature level of the liquid smoke toxicity properties. Based on these results it can be concluded that a). The production quality of liquid smoke is the best there is on the treatment of raw materials cinnamon at the level of a temperature of 400±10°C which shows the mortality rate of the Artemiasalina amounted to 19.048% is the smallest compared to the raw material to another, b) liquid smoke results of the combined treatment differences in raw materials (fiber coconut, coconut shell and cinnamon) with different temperature pyrolysis each show toxic properties (LC_{50}<30 ppm) with LC_{50} values respectively 14.9 ppm, 20.9 ppm and 20.5 ppm. c)the results of the combined treatment of liquid smoke raw materials (coconut fiber, coconut shell and cinnamon) with different concentrations of liquid smoke each show toxic properties (LC_{50}<30 ppm) with each LC_{50} value of 22.1 ppm, 19 6 ppm and 27 ppm. c) the results of the combined treatment .asap liquid pyrolysis temperature (100±10°C, 200±10°C, 300±10°C and 400±10°C) at different concentrations of liquid smoke each show toxic properties (LC_{50}<30 ppm) with LC_{50} values of each 20.5 ppm, 22 ppm, 15.9 ppm and 17.9 ppm. d) the results of the combined treatment liquid .asap differences in raw materials with different pyrolysis temperature at a concentration of 0 ppm, 12.5 ppm, 100 ppm, 500 ppm respectively each show toxic properties (LC_{50}<30 ppm) with LC50 values each 10.5 ppm, 11.6 ppm, 39.8 ppm, 18.6 ppm, 11.6 ppm while the concentration of 50 ppm and 1000 ppm LC_{50} values each at 55 ppm and 48.4 ppm are not toxic (LC_{50}> 30 ppm), subsequent regression line treatment combined with differences in raw materials pyrolysis temperature of the liquid smoke concentration of 50 ppm, 500 ppm and 1000 ppm have a weak relationship to the value of probit with r^2 values each at 0.1049, 0 , 2141 and 0.2308. while the other concentration of 0 ppm, 12.5 ppm, 50 ppm and 100 ppm have a stronger relationship with the probit value indicated by r^2 value respectively 0.7159, 0.8495, 0.807 and 0.8181.

Key words : type of raw material, temperature, liquid smoke, concentration, toxicity.

1.Introduction

According to [1], one test of bioactivity that is easy, fast, inexpensive and accurately by using larval shrimp Artemiasalina Leach. known as Brine Shrimp Lethality Test (BSLT). Shrimp larvae mortality test is one test method bioactivity in natural materials research compound. Use of shrimp larvae for the benefit of bioactivity studies have been conducted since 1956 and since then it has been a lot done on environmental studies, toxicity, and screening for bioactive compounds from the plant tissue. This test is a preliminary test to observe the pharmacological activity of a compound one of which is anti-cancer. The application for the bioactivity of the system by using the shrimp larvae, among others, to determine pesticide residues, local anesthetic, morpin derived compounds, mycotoxins, carcinogenicity compound and pollutants to the sea as well as an inexpensive alternative method for cytotoxicity assay [2]. The active compounds that have a high bioactivity known based on the value of 50% Lethal Concentration (LC_{50}), which is a value that indicates the concentration of toxic substances that can cause the death of test animals to 50%. Mortality data were obtained and processed by probit analysis formulated by [3] for determining the LC_{50} values at 95% confidence level. The chemical compound has the potential bioactive if it has LC_{50} values of less than 1,000 pg / ml [1].

BSLT test using larval shrimp Artemiasalina done incubate the eggs in seawater assisted by aeration. Artemiasalina eggs will hatch into larvae perfectly within 24 hours. A. salina larvae are well used to test BSLT is aged 48 hours because if more than 48 hours it is feared the death was not due to toxicity of Artemiasalina extract but by the limited supply of food[1,13]. The benefit of shrimp larvae of A. salina for this BSLT test is sensitive to the nature of the test material, the cycle time of life more quickly, easily bred and the price is cheap. A. salina sensitive nature likely caused by circumstances were very thin skin membrane that allows the diffusion of substances from the environment that affect the metabolism in the body. A. salina is found in almost all surface waters in the world who have a salinity range of 10 - 20g / L, it is this which causes easily bred. Newly hatched larvae are called nauplius oval and reddish color with a length of 400 μm with a weight of 15 g. Member body consisting of a pair of small antennae (or antenna anteluena I) and a pair of large antennae (antenna or antenna II). At the front of the two small antennae that are red spots that function as eyes (oselus). At the rear there is a pair of mandibular barbels magnitude (jaw) is small, whereas in the belly (ventral) side of the front there labrum[4].

Shrimp larvae toxicity test toxicity testing is one that is fast, safe, practical and economical for screening, fractionation, and determination of bioactivity of compounds of natural materials. National Cancer Institute of the United State of America (USA NCI) has found a significant relationship between toxicity testing on shrimp nauplius (Bhrine Shrimp Lethality Test) with inhibition of human tumor cells in vitro. According to [15] The major component of wood vinegar products are acetic acid, methanol, propanoic acid, phenolic and carbonyl compounds. The wood vinegar improves soil quantity eliminates pests, accelerating plant growth, plant growth regulator or growth inhabiting

Given waste plantation crops like coconut husk, coconut shell and cinnamon are widely available in the province of West Sumatra, and yet provide optimum added value, then it needs to be processed into liquid smoke. Research nature of the toxicity of liquid smoke of various kinds of raw materials and the temperature pyrolysis different has not been done in this study aimed to find out the nature of the toxicity of combined treatment of different types of raw materials (coconut fiber, coconut shell and cinnamon) with temperature pyrolysis different .

2.Materials and Methodology

Tools and instruments used in this study include tools laboratory glassware, test tube rack, aluminum foil, paper filter evaporator, vortex, desiccator, hot plate, aerator, fluorescent lights, 65 mesh sieve, oven, analytical balance (AND GH-202), blender, label paper, rulers, pencils, aluminum foil, plastic, filter paper, cotton, erlenmeyer flask, becker glass, measuring cups, funnels, test tubes, spatulas, stirring rod, a Pasteur pipette, glass bottles, incandescent lamps, weighing bottle, a measuring cup, a capillary tube, vial, micro pipette, magnifying glass, oven, and 1 set maker laboratory-scale liquid smoke [5]as the picture 1.

Materials and chemical reagents used in this study is a waste coconut husks, coconut shells obtained from Pasar Raya Padang and cinnamon already taken the outer skin is obtained from the farmers cinnamon in

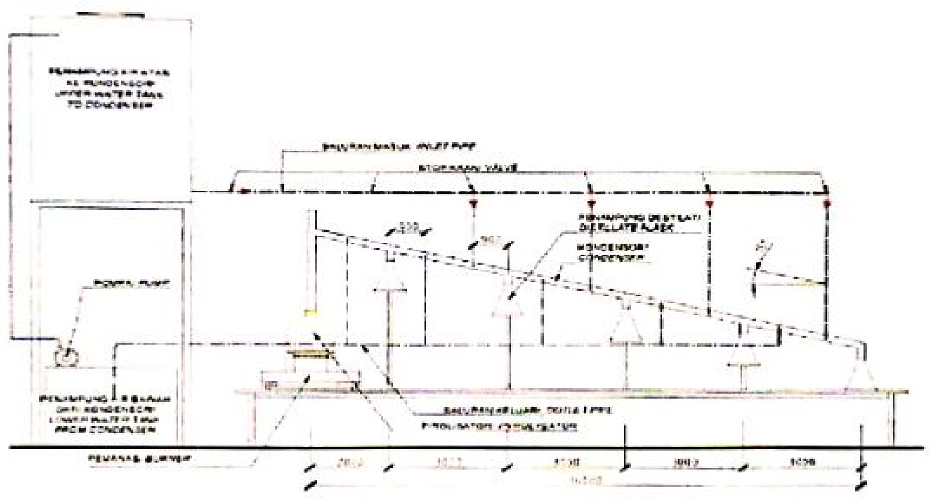
Tanah Datar. Artemiasalina, DMSO 50%, 70% ethanol , pro analysis methanol (Merck), distilled water, sea water.

2.1.Implementation Research

The stages of the implementation of this study consisted of three phases:

2.1.1.Make means of pyrolysis liquid smoke

Circuit extraction tool liquid smoke made at laboratory scale refers to the results of research and the characteristics of liquid smoke [5]. In this study using the tool maker of liquid smoke which comprises one unit of equipment condenser complete with water in the form of a drum capacity of 100 liters equipped with a water pump to help circulate cooling water along the 14 meter equipped with a hose for water circulation, place a container of liquid smoke in the form of a tube Erlenmeyer capacity of 500 ml amounted to 5 pieces, stainless stell kiln capacity of 3 kg and a stove burner LPG fueled at the end of the pipe as well as pyrolysis include a vacuum pump to draw the smoke of burning in order to obtain a liquid smoke as shown in Figure 1 below.



Gambar 1. Alat penghasil asap cair skala laboratorium.

Figure 1.Liquid smoke device of a laboratory scale

2.1.2. The process of pyrolysis (manufacture) of liquid smoke

Research on the manufacture of liquid smoke by means of pyrolysis refers to the above research activities to provide input to redesign the device of liquid smoke laboratory scale. After the tool maker of liquid smoke strung well then continued with producing liquid smoke. This process starts from raw material preparation providing coconut husk, coconut shell and cinnamon dry with each weighing about 40 kg with a moisture content ranging from 4-10%, cleaned of dirt. Raw materials in order to further cut its small size with uniform size ± 4- 9 cm2. Next activities include the raw materials into pyrolysis reactor during the five (5) hours with each weighing 3 kg sample at a temperature of 100± 10°C; 200 ±10°C; 300±10°C; 400±10°C. using fuel burner LPG stove. The water pump is used to drain water from the water source to the condenser. Burner and water pump switched on simultaneously. Container distillate (liquid smoke) accommodated using glass bottles. Measuring temperature using a thermometer and measurements were performed every ½ hour measured at several places, namely in pirolisator, container distillates, as well as water resources, as well as the inlet and outlet of the condenser. After 5 hours, will obtain three fractions, namely fraction of the solid form of carbon, in the form of tar heavy fraction and light fraction in the form of smoke and gas methan.Selanjutnya light fraction is passed kepipa condensation in order to obtain a liquid smoke while methane remained the gas and condensed.

Liquid smoke were then allowed to stand for one week, a new analysis. The purpose of the deposition during 1 (one) week to precipitate impurities that exist in liquid smoke. After 1 (one) week liquid smoke silenced cytotoxic further analysis.

2.1.3.Test cytotoxic liquid smoke method Brine Shrimp Lethality Test (BSLT) bioassay system [1,6] with the phases of work as follows:

a. Selection of eggs artemiasalina Leach

Selection of shrimp eggs made by soaking the eggs in distilled water for one hour. Good egg will sink whereas unfavorable egg will float.

b. Preparation of larvae Artemia Salina Leach

Preparation of shrimp larvae hatch shrimp done 48 hours before the test. Hatching eggs is done by immersing them in sea water sufficiently to illuminate the container that is not occupied by shrimp eggs with incandescent lights.

c. The division of the treatment group

In this study, shrimp larvae were divided into five treatment groups at random, namely[14]:

1. Group K is 10 larvae shrimp fed with a liquid smoke concentrations of 0 ug / ml.
2. The group P1 is 10 larvae shrimp fed liquid smoke with a concentration of 12.5 ug / ml in the media.
3. P2 group is 10 larvae shrimp fed liquid smoke with a concentration of 25 ug / ml in the media.
4. P3 group is 10 larvae shrimp fed with a liquid smoke concentration of 50 ug / ml in the media.
5. P4 group is 10 larvae shrimp fed with a liquid smoke concentration of 100 pg / ml in the media.
6. P5 group is 10 larvae shrimp fed liquid smoke with a concentration of 500 pg / ml in the media.
7. P6 group is 10 larvae shrimp fed liquid smoke with a concentration of 1000 pg / ml in the media.

d. Implementation of toxicity tests

Test execution is done by first equalizing the final volume of liquid smoke results from a combination of the three treatment materials (coco husk, coconut shell, cinnamon) with different pyrolysis temperature is a temperature of 100±10°C; 200±10°C; 300±10°C; and 400±10°C with a concentration ratio of treatment above is diluted by adding seawater advance into each test tube until liquid smoke above were mixed, then just put shrimp larvae that have been aged 48 hours in a series of test tubes containing liquid smoke which prepared respectively of 10 animals so that the volume in each tube to 5 ml. Test tube and then placed under incandescent light illumination for 24 hours, then counted the number of dead shrimp larvae. Standard criteria to assess mortality of shrimp larvae is when the shrimp larvae showed no movement during several seconds of observation.

e. Data collected

The data collected are primary data obtained from the amount of shrimp larvae died 24 hours after treatment in each combination of three (3) treatment of the type of raw material, pyrolysis temperature and concentration of different liquid smoke.

f. Hatching eggs artemiasalina

Artemia soaked in fresh water for 15-30 minutes. Then soaked in 10 liters of seawater. Hatching temperature is 25-30 ° C and pH ± ± 6-7. The eggs will hatch after 18-24 hours and the larvae are called nauplii. Nauplii ready to test BST after these larvae was 48 hours.

g. Test toxicity BST Extract Method

Liquid smoke results of the combined treatment of raw materials with different pyrolysis temperature taken 50 mg, each dissolved in 5 ml of methanol. Created dilution 1000, 500,100,50,25,12,5 and 0 ug/ml. Testing is done by inserting 10 larvae of Artemiasalina was 48 hours into glass jars already containing 1 ml solution of 4 ml of liquid smoke and seawater. After 24 hours, the number of dead larvae were calculated with the aid of a magnifying glass.

2.2.Experimental design

The study was conducted using a factorial experimental design 3 x 4 x 6 with three replications thus obtained 72 experimental units. A factor is the type of raw material comprising coconut husk, coconut shell and cinnamon, factor B level pyrolysis temperature of 100 ± 10°C; 200 ± 10° C; 300 ± 10°C; and 400 ± 10° C and factor C level liquid smoke concentration is 0 ppm, 12.5 ppm, 50 ppm, 100 ppm, 500 ppm and 1000 ppm. The parameters measured were the number of dead Artemia 50% of the total larvae test. Then calculated LC50 values by entering the numbers probit (50% larval mortality trials).

2.3.Data analysis

Toxicity effects of the observations was analyzed by percent of deaths.

$$\% \text{ Larva} = \frac{\text{The number of dead larvae}}{\text{Total larvae test}} \times 100\%$$

By knowing the mortality of larvae Artemiasalina, then searched through tables and figures made probit equation:

$$Y = Bx + A$$

where Y = log concentration, and X = Score probit

Furthermore, the numbers sought probitprobit through tables and graphs created with log concentration as the x-axis against the percentage of mortality in probit units as the y-axis. LC50 value is the concentration of a substance which causes the death of 50% of test animals obtained by using the linear regression equation $y = a + bx$. A substance is said to be active or toxic substances when the LC50 value of less than 1000 ppm to extract and less than 30 ppm of a compound [7].

3.Results and Discussion

3.1..Effect combined treatment of raw materials with different temperature pyrolysis gainst the percentage of mortality, probit values and LC_{50}

Results of analysis of variance (%) mortality showed an interaction between the combined treatment differences raw material liquid smoke with a temperature pyrolysis different to the value of cytotoxic (%) mortality liquid smoke ($P<0.05$), as well as in combination treatment differences in raw materials with a concentration of smoke different liquid while the combined treatment of the pyrolysis temperature difference with different concentrations showed no interaction ($P>0.05$) as well as on a combined treatment of raw materials, pyrolysis temperature and concentration showed no interaction ($P>0.05$).

On the results of variance probit shows the interaction between the combined treatment differences raw material liquid smoke with a temperature pyrolysis different to the value of cytotoxic (%) mortality liquid smoke ($P<0.05$), as well as in combination treatment differences in raw materials with the concentration of liquid smoke different while for the combined treatment of the difference in temperature pyrolysis with different concentrations showed no interaction ($P>0.05$) as well as on a combined treatment of raw materials, temperature pyrolysis and concentration showed no interaction ($P>0.05$) the observation of the average results of toxicity tests (%) mortality liquid smoke given treatment raw materials with different pyrolysis temperatures presented in Table 1.

Table 1. Observations average (%) mortality in liquid smoke toxicity tests are given treatment of raw materials with different temperature pyrolysis.

Factor	Temperature Pyrolisis (°C) (T)				Mean (T)	Interaktion
And Level	100 (T_1)	200(T_2)	300 (T_3)	400(T_4)		(T x B)
Coconut husk(B_1)	46.19± 4.52 ^{ab}	47.143± 4.7 ^{ab}	49.048±4.71 ^{ab}	52.381± 4.65 ^{a}	48.69± 4.57 ^{c}	4.03
Coconut shell (B_2)	44.286± 4.44 ^{bcd}	44.286± 4.46 ^{bcd}	40 ± 4.26 ^{cde}	38.971 ± 4.65 ^{de}	41.89± 4.24 ^{b}	8.87
Cinnamon (B_3)	34.286 ± 4.39 ^{ef}	31.905 ± 4.00 f^{g}	26.667 ± 3.17 ^{g}	19.048 ± 2.39 ^{h}	27.98± 3.61 ^{a}	11.28
Mean raw material (B)	41.59± 4.45 ^{a}	41.11± 4.29 ^{a}	38.57± 4.25 ^{ab}	36.80± 4.23 ^{b}	39.52	
Interaktion (B xT)	6.67	8.25	8.89	13.28		

* Different superscript letters in rows and columns show the average significant difference (P <0.05)

Based on Table 1 shows the value of a positive interaction both at treatment differences in raw materials with different pyrolysis temperature (line) and the pyrolysis temperature treatment with the types of raw materials (columns). This means that both factors influence both the raw materials and the pyrolysis temperature to respond jointly to the (%) mortality brine leach very large, compared to the treatment of each factor. BSLT toxicity test method is acute toxicity test where the toxic effect of a compound is determined in a short time, which ranges up to 24 hours after administration of the test dose [1]. BSLT method chosen because it is one of the methods bioactivity that is easy, fast, cheap and accurate. This method is often used to determine the toxicity of the nature / botanical extracts as well as for screening compounds for their anticancer positive correlation between the methods BSLT with cytotoxic test using cancer cell cultures [8].

In Table 1 shows the average% mortality of Artemiasalina on raw materials cinnamon decreases with increase in temperature pyrolysis. The average percentage mortality raw materials cinnamon on pyrolysis temperature of 400 ± 10°C showed the lowest number 19.048%, and the highest percentage rate of Artemiasalina deaths occur in raw material coco on pyrolysis temperature of 400 ±10°C of 52.531%. This means that liquid smoke cinnamon has properties of lower mortality compared with the raw material coconut shells and coconut fiber. The nature of low mortality means that the toxic effects are present in such liquid smoke cinnamon aldehyde compounds, ketones, phenols provide less toxic effects. it is indicated by the decline in the average mortality rate of Artemiasalina. An increase in the pyrolysis temperature showed a decline in the percentage of deaths allegedly caused by Artemia higher pyrolysis temperatures will cause more and more compounds are formed, and also it will be many compounds present in the liquid smoke is lost, causing less toxic effects.

The observation of the average results of toxicity tests (Probit) given liquid smoke treatment of raw materials with different temperature pyrolysis can be seen in Table 2 below.

Table 2. Observations average Probit on liquid smoke toxicity tests are given treatment of raw materials with different temperature pyrolysis.

Factor	Temperaturepyrolisis(oC) (T)				Mean (T)	interaktion (T*B)
And Level	100 (T_1)	200(T_2)	300 (T_3)	400(T_4)		
Coconut husk(B_1)	4.90± 4.23 ^{d}	4.93± 4.23 ^{c}	4.97± 4.23 ^{b}	5.06± 4.23 ^{a}	4.97± 4.58 ^{a}	0.09
Coconut shell (B_2)	4.86± 4.23 ^{e}	4.86± 4.23 ^{e}	4.75± 4.23 ^{e}	4.72± 4.23 ^{e}	4.80± 4.24 ^{b}	-0.03
Cinnamon (B_3)	4.59± 4.23 ^{f}	4.53± 4.23 ^{f}	4.38± 4.23 ^{f}	4.12± 4.23 ^{f}	4.41± 3.61 ^{c}	-0.26
Mean raw material (B)	4.78± 4.44 ^{a}	4.77± 4.43 ^{a}	4.70± 4.25 ^{ab}	4.63± 4.32 ^{c}	4.72	
Interaktion (B xT)	-0.04	0.053	-0.09	0.14		

* Different superscript letters in rows and columns show the average significant difference (P <0.05)

Table 2 shows the value of positive interaction in the treatment of coconut coir raw materials at different pyrolysis temperature (line) whereas the raw material coconut shell and cinnamon at different pyrolysis temperature indicates the value of negative interactions. Then on the combined treatment of the pyrolysis temperature of 100°C and 300°C on different raw materials shows the value of negative interaction, it is different to the treatment temperature of 200°C to 400°C with different types of raw materials level values are negative. If the value of positive interaction means that both factors influence both the raw materials and the pyrolysis temperature to respond jointly to the probit value, compared to the treatment of each factor. Furthermore, the value of negative interaction means on both factors give the opposite response or the response given to the second factor probit value lower than the performance on each factor. Furthermore, the LC_{50} value in the combination treatment of raw materials with different pyrolysis temperature in Table 3 below.

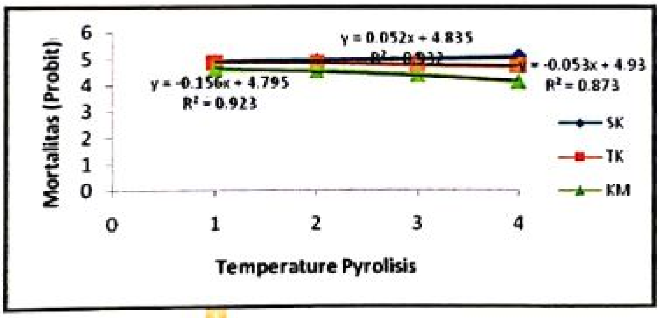
Table 3.Observations on the average LC_{50} toxicity tests given liquid smoke treatment of raw materials with different temperature pyrolysis.

Factor	Temperature pyrolisis (°C) (T)				Mean (T)	Interaction (T*B)
And Level	100 (T_1)	200(T_2)	300 (T_3)	400(T_4)		
Coconut husk(B_1)	14.9	14.9	14.9	14.9	14.9 c	-
Coconut shell (B_2)	20.9	20.9	20.9	20.9	20.9 a	-
Cinnamon (B_3)	20.5	20.5	20.5	20.5	20.5 b	-
Mean raw material (B)	18.76	18.76	18.76	18.76	18.76	
Interaction (B xT)	3.73	3.73	3.73	3.73		

* Different superscript letters in rows and columns show the average significant difference ($P < 0.05$)

Based on Table 3 shows the value of positive interaction (3.73) on a column combination of pyrolysis temperature treatment on the raw materials of different liquid smoke to the LC50. It means both treatment factors together give a strong response to the results LC50 liquid smoke. The results that the average LC50 in the respective pyrolysis temperature of 18.76 ppm, meaning to kill 50 percent of arrhythmia requires salina leach liquid smoke as much as 18.76 ppm. Furthermore, LC 50 value in the coconut husk, coconut shell and cinnamon respectively at 14.9 ppm, 20.9 ppm and 20.5 ppm.

According to Table 28 and 29 that BSLT toxicity tests done by determining the LC_{50} value of the activity of the active components of the plant against the larvae of Artemiasalina Leach. An extract is said to be toxic by the methods BSLT if the extract can kill 50% of test animals at concentrations of less than 1000 ppm [1]. For images regression line relationship type of raw material liquid smoke with pyrolysis temperature differences in mortality probit value as figure 2 below.



Note: 1 = temperature of 100 ± 10 ° C; 2 = temperature of 200 ± 10 ° C; 3 = temperature of 300 ± 10 ° C; 4 = temperature of 100 ± 10 ° C

Figure 2. Mean mortality Probit value of some type of raw materials with different Pyrolysis Temperature Level

Toxicity tests conducted on a combination of coconut husks at different pyrolysis temperature shows LC_{50} value of 14.9 ppm, and coconut shell at different pyrolysis temperature of 20.9 ppm, and cinnamon in a different pyrolysis temperature of 20.5 ppm. This indicates that the compound liquid smoke of the three different feedstocks has the potential acute toxicity by BSLT method and can be developed as anticancer because LC_{50}<30 ppm (Juniarti et.al. 2005). Based on regression analysis that a combination of differences in raw materials (coconut fiber, coconut shell and cinnamon) shows a close relationship to the value of probit with r2 values respectively 0.9324, 0.8737 and 0.9239. Potential acute toxicity possessed liquid smoke is influenced by the content of secondary metabolites which owned the extract. The presence of the flavonoid extract in the cell environment, causing the OH groups in flavonoids bind to the cell membrane integral proteins. This causes terbendungnya active transport of Na + - K +. Active transport stop influx of Na + ions causes uncontrolled into the cells, causing rupture of the cell membrane (Scheuer, 1994). Rupture of cell membranes that causes the death of larvae of Artemiasalina.

3.2.Effect raw materials combined treatment with different concentrations of liquid smoke the percentage of mortality, probit values and LC_{50}

The observation of the average liquid smoke toxicity test results are given raw material treatment with different concentrations of liquid smoke to (%) mortality of Artemia in Table 4 below.

Table 4. Observations average liquid smoke toxicity test results are given raw material treatment with different concentrations of liquid smoke to (%) mortality of Artemia.

Factor	Consentrationliquid smoke (ppm)							Mean (T)	Interaction
And Level	0	12.5	25	50	100	500	1000		(T*B)
Coconut husk(B_1)	3.3 ± 4.9 ^{i}	22.5 ± 7.5 ^{f}	26.67 ± 7.8 ^{f}	49.17 ± 9.9 ^{de}	66.67 ± 9.8 ^{bc}	72.5 ± 6.2 ^{b}	100 ± 0.0 ^{a}	25.42 ± 4.57 ^{a}	-40.12
Coconut shell (B_2)	3.3 ± 3.5 ^{i}	16.7 ± 1.4 ^{fgh}	20.83 ± 1.4 ^{fg}	39.17 ± 0.8 ^{e}	56.67 ± 2.6 ^{cd}	63.33 ± 2.2 ^{bc}	92.5 ± 1.3 ^{a}	20.0 ± 4.24 ^{b}	-37.78
Cinnamon (B_3)	2.5 ± 1.3 ^{i}	5.83 ± 1.5 ^{hi}	10 ± 1.2 ^{ghi}	20 ± 2.1 ^{fg}	41.67 ± 3.2 ^{e}	48.33 ± 3.9 ^{de}	67.5 ± 5.5 ^{bc}	9.58 ± 3.61 ^{c}	-29.68
Mean raw material (B)	3.05 ± 4.67 ^{a}	15.00 ± 9.10 ^{b}	19.17 ± 8.7 ^{b}	36.11 ± 15.17 ^{c}	55.00 ± 14.24 ^{d}	61.39 ± 13.76 ^{a}	86.67 ± 17.88 ^{f}	18.33	
Interaction (B xT)	0.55	7.23	7.22	12.78	10.00	10.00	16.67		

* Different superscript letters in rows and columns show the average significant difference (P <0.05)

Based on Table 4 shows the value of a negative interaction at treatment concentrations of smoke with different types of raw materials (row) while on treatment combination of raw materials with different concentrations of liquid smoke indicates the value of positive interaction. If the value of positive interaction means that both factors influence both the raw materials and the concentration of liquid smoke to respond jointly to the value (%) mortality, compared to the treatment of each factor. Furthermore, the value of negative interaction means on both factors give the opposite response or the response given to the value of the second factor (%) mortality was lower than the performance on each factor. The observation of the average liquid smoke toxicity test results are given treatment of raw materials with different temperature pyrolysis against Probit value in Table 5 below.

Table 5. The observation of the average liquid smoke toxicity test results are given raw material treatment with different concentrations of liquid smoke to the probit value.

Factor	(K) Consentrationliquid smoke (ppm)							Mean (T)	Interaction
And Level	0(K_0)	12.5(K_1)	25(K_2)	50 (K_3)	100(K_4)	500(K_5)	1000(K_4)		(T*B)
Coconut husk(B_1)	3.16± 2.39^{h}	4.24± 2.39^{h}	4.38± 2.39^{h}	4.98± 2.39^{h}	5.43± 2.39^{h}	5.59± 2.39^{h}	8.72± 2.39^{h}	5.21± 2.39^{h}	2.48
Coconut shell (B_2)	3.16± 2.39^{h}	4.03± 2.39^{h}	4.19± 2.39^{h}	4.74± 2.39^{h}	5.17± 2.39^{h}	5.34± 2.39^{h}	6.44± 2.39^{h}	4.72± 2.39^{h}	1.78
Cinnamon (B_3)	3.04± 2.39^{h}	3.43± 2.39^{h}	3.72± 2.39^{h}	4.16± 2.39^{h}	4.79± 2.39^{h}	4.96± 2.39^{h}	5.45± 2.39^{h}	4.22± 2.39^{h}	1.55
Mean raw material (B)	3.12± 2.39^{h}	3.9± 2.39^{h}	4.10± 2.39^{h}	4.63± 2.39^{h}	5.13± 2.39^{h}	5.30± 2.39^{h}	6.87± 2.39^{h}	4.72	
Interaction (B xT)	-0.08	-0.54	-0.44	-0.55	-0.43	-0.42	-2.18	-0.66	

Explanation of: different superscript letters in rows and columns show the average significant difference ($P < 0.05$)

Based on Table 5 shows the value of positive interaction in the combined treatment of different types of raw materials with different pyrolysis temperature (line), while the concentration of liquid smoke treatment combination with different types of raw materials showed a value of negative interactions. If the value of positive interaction means that both factors influence both the raw materials and the concentration of liquid smoke to respond jointly to the Probit values, compared to the treatment of each factor. Furthermore, the value of a negative interaction means on both factors give the opposite response or the response given to the second factor probit value lower than the performance on each factor. Furthermore, LC 50 values in the combined treatment of treatment of raw materials with different concentrations of liquid smoke to the probit value contained in Table 6 below.

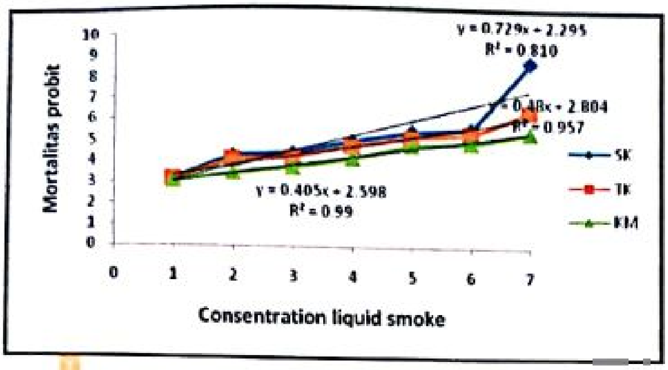
Table 6. The observation of the average liquid smoke toxicity test results are given raw material treatment with different concentrations of liquid smoke to the LC50 value.

Factor	(K) Consentrasiliquid smoke (ppm)							Mean (T)	Interaction
And Level	0(K0)	12.5(K1)	25(K2)	50 (K3)	100(K4)	500(K5)	1000(K6)		(T*B)
Coconut husk(B_1)	22.1	22.1	22.1	22.9	22.1	22.1	22.1	22.1 b	--
Coconut shell (B_2)	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6 a	-
Cinnamon (B_3)	27	27	27	27	27	27	27	27 c	
Mean raw material (B)	22.9	22.9	22.9	22.9	22.9	22.9	22.9	22.9	-
Interaktion (B xT)	3.26	3.26	3.26	3.26	3.26	3.26	3.26		

* Different superscript letters in rows and columns show the average significant difference ($P < 0.05$)

Based on Table 6 shows the value of positive interaction (3:26) on a column of liquid smoke concentration treatment combinations on the different raw materials to the LC50. It means both treatment factors together give a strong response to the results LC50 liquid smoke. The results that the average LC50 in the respective pyrolysis temperature of 22.9 ppm, meaning to kill 50 percent of arrhythmia requires salina leach liquid smoke as much as 22.9 ppm. Furthermore, LC 50 value in the coconut husk, coconut shell and cinnamon each at 22.1 ppm, 19.6 ppm and 27 ppm.

In Table 6 shows the phenomenon that the higher the concentration of liquid smoke on the third raw materials used in making liquid smoke tendency of an increase in the percentage of deaths Artemiasalina. This means that the higher the concentration of liquid smoke is used then the concentration of liquid smoke will become increasingly concentrated, so the percentage of mortality of Artemiasalina also be increased. For the third raw material used was that the raw material liquid smoke with cinnamon showed the highest percentage of Artemiasalina lowest mortality when compared to materials coconut husk and coconut shell. For images regression relationship liquid smoke kinds of raw materials with different concentrations of liquid smoke on mortality probit values such as figure 3 below.



Note: 1 = 0ppm, 2 = 12.5ppm, 3 = 25ppm, 4 = 50ppm, 100ppm = 5, 6 = 500ppm, 7 = 1000ppm

Figure 3. Mean mortality Probit value of some type of raw material with liquid smoke concentration levels of different

Toxicity tests conducted on liquid smoke of different raw materials with different concentrations of liquid smoke showed LC_{50} values ranging from 19.6 ppm - 27 ppm. This shows that the liquid smoke of the three materials have the potential acute toxicity by BSLT method and can be developed as anticancer ieLC_{50}value<30 ppm [7]. Based on the regression line that combined treatment with different types of raw materials concentrations all showed a strong relationship with R2 each at 0.8102. 0.9577 and 0.99. The appearance of the image regression line each show increases in value when the concentration of liquid smoke probit raised. It is alleged by the higher concentration of liquid smoke, the more there are compounds such as aldehydes, ketones and phenols, causing the value of probit tendency to rise. In addition to flavonoids, there are some secondary metabolites are present in 70% ethanol extract. The compounds are secondary metabolites include saponins and glycosides. Such compounds can act as stomach poisoning or stomach poison. Therefore, when these compounds into the body of larvae, larval digestive system will be disrupted. In addition, these compounds inhibit the taste receptors in the mouth of the larvae. This resulted in the larvae fail to get a taste stimulus that can not recognize the food. As a result, the larvae die of starvation [9,10].

3.3.Effect combination of liquid smoke concentration treatments with different temperature pyrolysis the percentage of mortality, probit values and LC $_{50}$

The observation of the average liquid smoke toxicity test results are given treatment pyrolysis temperature and concentration of different liquid smoke against (%) mortality of Artemia can be seen in Table 7 below.

Table 7. The observation of the average results of toxicity tests were given treatment liquid smoke liquid smoke concentration and temperature of different pyrolysis against (%) mortality of Artemia.

Concentration liquid smoke	Temperature pyrolisis (°C) (T)				Mean
(K)	100 + 10 °C	200 + 10 °C	300 + 10 °C	400 + 10 °C	(T)
0 ppm	3.333 ± 5.0	3.333 ± 5.0	3.333 ± 5.0	2.222 ± 4.41	3.055
12.5 ppm	15.556 ± 8.82	14.444 ± 8.82	14.444 ± 8.82	15.556 ± 11.3	15.000
25 ppm	20 ± 7.07	18.889 ± 9.28	18.889 ± 7.81	18.889 ± 15.89	19.167
50 ppm	36.667 ± 15.81	36.667 ± 13.22	35.556 ± 15.89	35.556 ± 18.11	36.112
100 ppm	56.667 ± 10.0	58.889 ± 10.54	53.333 ± 15.0	51.111 ± 20.76	55.000
500 ppm	65.556 ± 8.82	64.444 ± 10.14	53.333 ± 15	51.111 ± 20.76	58.611
1000 ppm	93.333 ± 7.07	91.111 ± 10.54	84.444 ± 19.44	77.778 ± 26.35	86.667
Rata-rata (T)	41.59a	41.11a	37.62b	36.03c	39.09

Description: Different superscript letters in rows and columns show the average significant difference (P <0.05)

Based on the data the average percentage of dead Artemiasalina in Table 33 showed an increase in the percentage of deaths Artemia with higher pyrolysis temperature and concentration of liquid smoke is used. Percentage of Artemiasalina mortality is highest in the combined treatment of liquid smoke concentration of 1000 ppm in the pyrolysis temperature of 100 ± 100 ° C of 93.33% is not significantly different from 1000 ppm concentration of liquid smoke on the pyrolysis temperature of 200 ± 100 ° C, while the lowest value Artemiasalina deaths occur in combination treatment without the administration of liquid smoke at a temperature of 400 ± 100 ° C. This is caused by the higher temperatures used pyrolysis followed by the use of liquid smoke concentration the higher the percentage of deaths will increase the rate of Artemia. This condition means that the mortality effect of liquid smoke cinnamon will increase as the concentration of liquid smoke and temperature pyrolysis used.

Further observations of the average liquid smoke toxicity test results are given treatment pyrolysis temperature and concentration of different liquid smoke to the Probit values can be seen in Table 8 below.

Table 8. The observation of the average results of toxicity tests were given treatment liquid smoke liquid smoke concentration and temperature of different pyrolysis against probit value.

Conc. liquid smoke	Temperature pyrolisis (oC) (T)				Mean
(K)	100 + 10 oC	200 + 10 oC	300 + 10 oC	400 + 10 oC	(T)
0 ppm	3.16	3.16	3.16	2.98	3.115 d
12.5 ppm	3.99	3.94	3.94	3.99	3.965 d
25 ppm	4.16	4.12	4.12	4.12	4.130 c
50 ppm	4.66	4.66	4.63	4.63	4.645 c
100 ppm	5.17	5.23	5.08	5.03	5.128 b
500 ppm	5.4	5.37	5.08	5.03	5.220 b
1000 ppm	6.49	6.35	6.01	5.77	6.155 a
Mean (T)	4.72	4.69	4.57	4.51	4.62

Description: Different superscript letters in rows and columns show the average significant difference (P <0.05)

According to Table 8 shows that there is no interaction combined treatment of liquid smoke concentration and temperature of different pyrolysis against probit value. This means that the concentration factor and temperature pyrolysis liquid smoke to respond separately do not do together. Probit value of 1000 ppm give the greatest probit 6.155 ppm means that the concentration of liquid smoke that the higher the ability to kill arrhythmia salina will be higher. Furthermore, based on figures obtained at the pyrolysis temperature rise does not show any increase in the value of probit.

Furthermore, the LC_{50} values at treatment combination treatment concentration and temperature pyrolysis liquid smoke differently to probit values. in Table 9 below.

Table 9. The observation of the average results of toxicity tests were given treatment liquid smoke liquid smoke concentration and temperature of different pyrolysis against LC_{50} values.

Concentration Liquid smoke	Temperature pyrolisis (°C) (T)				Mean
(K)	100 + 10 oC	200 + 10 oC	300 + 10 oC	400 + 10 oC	(T)
0 ppm	20.50	22.00	15.90	17.90	19.08
12.5 ppm	20.50	22.00	15.90	17.90	19.08
25 ppm	20.50	22.00	15.90	17.90	19.08
50 ppm	20.50	22.00	15.90	17.90	19.08
100 ppm	20.50	22.00	15.90	17.90	19.08
500 ppm	20.50	22.00	15.90	17.90	19.08
1000 ppm	20.50	22.00	15.90	17.90	19.08
Mean (T)	20.50 b	22.00 a	15.90 d	17.90 c	19.08

Description: Different superscript letters in rows and columns show the average significant difference (P <0.05)

		500 ppm	2.69	70±0.00	5.424	11.6
		1000 ppm	3	100±0.00	8.719	48.4
	300 + 10oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	20±10.0	4.158	11.6
		25 ppm	1.398	26.67±5.77	4.375	39.8
		50 ppm	1.699	53.33±5.77	5.082	55
		100 ppm	2	66.67±15.28	5.431	18.6
		500 ppm	2.69	73.33±8.77	5.622	11.6
		1000 ppm	3	100±0.00	8.719	48.4
	400 + 10oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	26.67±5.77	4.375	11.6
		25 ppm	1.398	30±10.0	4.476	39.8
		50 ppm	1.699	56.67±5.77	5.168	55
		100 ppm	2	73.33±5.77	5.622	18.6
		500 ppm	2.69	76.67±5.77	5.726	11.6
		1000 ppm	3	93.33±0.00	6.498	48.4
Coconut shell	100 + 10 oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	16.67±5.77	4.034	11.6
		25 ppm	1.398	20±0.00	4.158	39.8
		50 ppm	1.699	50±10.0	2	55
		100 ppm	2	60±10.0	5.253	18.6
		500 ppm	2.69	66.67±5.77	5.431	11.6
		1000 ppm	3	93.33±5.77	6.498	48.4
	200 + 10oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	16.67±5.77	4.034	11.6
		25 ppm	1.398	23.33±5.77	4.271	39.8
		50 ppm	1.699	40±10.0	4.747	55
		100 ppm	2	63.33±5.77	5.082	18.6
		500 ppm	2.69	70±0.00	5.253	11.6
		1000 ppm	3	93.33±5.77	6.498	48.4
	300 + 10oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	16.67±5.77	4.034	11.6
		25 ppm	1.398	20±0.00	4.158	39.8
		50 ppm	1.699	33.33±5.77	4.568	55
		100 ppm	2	53.33±5.77	5.082	18.6
		500 ppm	2.69.	60±10.0	5.253	11.6
		1000 ppm	3	93.33±5.77	6.498	48.4
	400 + 10oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	16.67±5.77	4.034	11.6
		25 ppm	1.398	20±0.00	4.158	39.8
		50 ppm	1.699	33.33±5.77	4.568	55
.		100 ppm	2	50±10.00	2	18.6
		500 ppm	2.69	56.67±5.77	5.168	11.6
		1000 ppm	3	90±0.00	6.282	48.4
Cinnamon	100 + 10 oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	6.67±5.77	3.501	11.6
		25 ppm	1.398	13.33±5.77	3.888	39.8
		50 ppm	1.699	20±10.00	4.158	55
		100 ppm	2	50±10.00	2	18.6
		500 ppm	2.69	60±10.00	5.253	11.6
		1000 ppm	3	86.67±5.77	6.112	48.4
	200 + 10oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	6.67±5.77	3.501	11.6
		25 ppm	1.398	10±0.00	3.718	39.8
		50 ppm	1.699	23.33±5.77	4.271	55
		100 ppm	2	46.67±5.77	4.917	18.6
		500 ppm	2.69	53.33±11.55	5.082	11.6
		1000 ppm	3	80±10.00	5.842	48.4
	300 + 10oC	0 ppm	0	3.33±5.77	3.162	10.5
		12.5 ppm	1.097	6.67±5.77	3.501	11.6
		25 ppm	1.398	10±0.00	3.757	39.8
		50 ppm	1.699	20±10.00	4.158	55
		100 ppm	2	40±10.00	4.748	18.6
		500 ppm	2.69	46.67±11.55	4.917	11.6
		1000 ppm	3	60±10.00	5.253	48.4

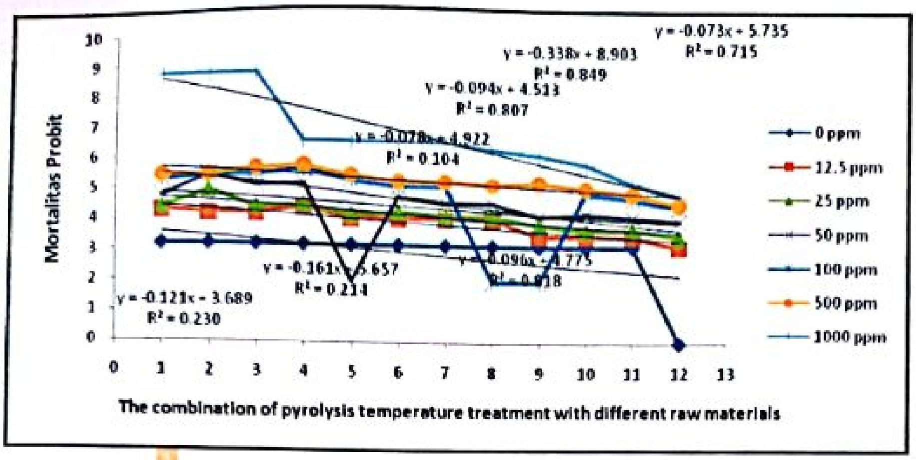
	400 + 10oC	0 ppm	0	0±0.00	0	10.5
		12.5 ppm	1.097	3.33±5.77	3.162	11.6
		25 ppm	1.398	6.67±5.77	3.501	39.8
		50 ppm	1.699	16.67±5.77	4.034	55
		100 ppm	2	30±10.00	4.476	18.6
		500 ppm	2.69	33.33±5.77	4.568	11.6
		1000 ppm	3	43.33±5.77	4.831	48.4

Description: * Different superscript letters in columns averaging showed significant difference (P <0.05)

Table 10 shows that the combined treatment of the raw material, pyrolysis temperature and different concentrations of liquid smoke in the trial, which was not significantly different effect on mortality of larvae of A. salina Leach. The number of larvae per cup test is 10 cows and concentration each performed three repetitions. The total number of larvae of A. salina Leach used was 2520 larvae. Larvae were used was 48 hours, because at this age are already full members of the larval body than when the larvae hatch. In observing the growth and development of larvae until the toxicity testing of the extract, use tools to observe that the magnifying glass.

Based on data from Table 10 that the greater the tendency or high concentrations of liquid smoke used for each raw material, the higher the response or the impact caused the death of test animals. Mortality and survival in a time period of exposure is a specific effect in acute toxicity tests with long-term exposure. Data from the test are quantal significant lethality test animals alive or dead. According to [12]Oral administration of ethanol extract at the highest doseof 18,000 mg/kg resulted in no mortalities or evidence of adverse effects, implying that *LD 50 >15.000 mg/kgbb which shown that extract is non toxic*

Based on the data the average percentage of dead Artemiasalina on a combination of three treatments (raw material, pyrolysis temperature and concentration of different liquid smoke) showed an increase in the percentage of deaths Artemia with the high concentration of liquid smoke is used. This is caused by the higher consumption of liquid smoke concentration will increase the rate of mortality percentage Artemia. This condition means that the mortality effect of liquid smoke cinnamon will increase as the concentration of liquid smoke and temperature pyrolysis used. Furthermore, from Table 10 shows the higher concentration of liquid smoke that is given to the larvae, a trend showing the higher mortality rate of larvae. This happened to the third raw material liquid smoke at different pyrolysis temperature. Liquid smoke coco with different pyrolysis temperature showed the highest mortality rate of larvae at each concentration after one hour, and subsequent liquid smoke liquid smoke coconut shell and cinnamon. Potential acute toxicity possessed liquid smoke is influenced by the content of secondary metabolites which owned the liquid smoke. The presence of the flavonoid extract in the cell environment, causing the OH groups in flavonoids bind to the cell membrane integral proteins. This causes terbendungnya active transport of Na + - K +. Active transport stop influx of Na + ions causes uncontrolled into the cells, causing rupture of the cell membrane [11]. Rupture of cell membranes that causes the death of Artemia larvae mortality percentage Artemiasalina.Untuk looking up to the level of 50%, then it will use the LC_{50} (lethal consentratian) means that this figure will be useful to predict the concentration of liquid smoke used until the death of Artemiasalina as much as 50% . The following was delivered value probit mortality Artemia to seek LC $_{50}$ contained in Figure 5 below.



Description 1 = SKT1,2 = SKT2,3 = SKT3,4 = SKT4, 5 = TKT1,6 = TKT2,7 = TKT3,8 = TKT4, 9 = KMT1, 10 = KMT2, 11 = KMT3, 12 = KMT4.

Figure 5.. Mean mortality Probit value of some raw materials to the pyrolysis temperature and the concentration of different liquid smoke

LC_{50} value on the combined treatment of differences in raw materials with different pyrolysis temperature at a concentration of 0 ppm, 12.5 ppm, 50 ppm, 100 ppm, 500 ppm and 1000 ppm 10.5 ppm, 11.6 ppm, 39.8 ppm, 55 ppm, 18.6 ppm, 11.6 ppm and 48.4 ppm. Based on the opinion of Juniarti et al. (2005) that the percentage data Artemiasalina leach mortality (Table 5) and LC_{50} values that the combination of raw materials to the pyrolysis temperature at a concentration of 0 ppm, 12.5 ppm, 50 ppm and 500 ppm are active for $LC_{50}<50$ and a concentration of 50 ppm, 100 ppm and 1000 ppm are not active. The data showed a trend the higher the concentration of liquid smoke is used more and more percentage of mortality of Artemia. The number of dead Artemia comparable with the high concentration of liquid smoke is used. It is alleged by the higher concentration of liquid smoke used to be a growing number of aldehyde and phenol compounds are formed, causing a growing number of dead Artemia. Furthermore, based on 15 images showed that the toxicity tests probit value indicated by the combined treatment differences in raw materials by the pyrolysis temperature on liquid smoke concentration of 50 ppm, 500 ppm and 1000 ppm have a weak relationship to probit. Value is indicated by the value of r^2 each at 0.1049, 0.2141 and 0.2308. For other concentration of 0 ppm, 12.5 ppm, 50 ppm and 100 ppm has a close relationship with the probit value indicated by the value of r^2 each at 0.7159, 0.8495, 0.807 and 0.8181. In addition to flavonoids, there are some secondary metabolites are present in liquid smoke. The compounds are secondary metabolites, among others allegedly are saponins and glycosides. Such compounds can act as stomach poisoning or stomach poison. Therefore, when these compounds into the body of larvae, larval digestive system will be disrupted. In addition, these compounds inhibit the taste receptors in the mouth of the larvae. This resulted in the larvae fail to get a taste stimulus that can not recognize the food. As a result, the larvae die of starvation [9,10].

4. Conclusion

1. The quality of the best production of liquid smoke contained in the raw material cinnamon treatment at a temperature of 400±10°C level which demonstrate against Artemiasalina mortality rate of 19.048% which is smaller than the second most other raw materials.
2. Liquid Smoke result of a combination treatment of differences in raw materials (coconut fiber, coconut shell and cinnamon) with different temperature pyrolysis each show toxic properties ($LC_{50}<30$ ppm) with LC_{50} value of 14.9 ppm respectively, 20, 9 ppm and 20.5 ppm.
3. Liquid Smoke result of a combination treatment of the raw material (coconut fiber, coconut shell and cinnamon) with different concentrations of liquid smoke each show toxic properties ($LC_{50}<30$ ppm) with each LC_{50} value of 22.1 ppm, 19 6 ppm and 27 ppm.

4. Liquid Smoke mixed results pyrolysis temperature treatment (100 ± 10°C, 10°C ± 200, 300 ±10°C and 400±10°C) at different concentrations of liquid smoke each show toxic properties (LC_{50}<30 ppm) with LC_{50}values respectively 20.5 ppm, 22 ppm, 15.9 ppm and 17.9 ppm.
5. Liquid Smoke result of a combination treatment of differences in raw materials with different pyrolysis temperature at a concentration of 0 ppm, 12.5 ppm, 100 ppm, 500 ppm respectively each show toxic properties (LC_{50}<30 ppm) with LC_{50}values of each of 10.5 ppm, 11.6 ppm, 39.8 ppm, 18.6 ppm, 11.6 ppm while the concentration of 50 ppm and 1000 ppm LC_{50}values each at 55 ppm and 48.4 ppm are not toxic (LC_{50}> 30 ppm), subsequent regression line treatment combined with differences in raw materials pyrolysis temperature of the liquid smoke concentration of 50 ppm, 500 ppm and 1000 ppm have a weak relationship to the value of probit with R2 values of 0.1049 respectively, 0. 2141 and 0.2308, while the other concentration of 0 ppm, 12.5 ppm, 50 ppm and 100 ppm have a stronger relationship with the probit value indicated by the value of r^2 each at 0.7159, 0.8495, 0.807 and 0.8181.

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